Early histological, microbiological, radiological, and clinical response to cemented and screw-retained all-ceramic single crowns

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Abstract: OBJECTIVES To assess the early histological, microbiological, radiological, and clinical response to cemented and screw-retained all-ceramic single-tooth implant-supported reconstructions. MATERIALS AND METHODS Patients with single-tooth implants were randomly allocated to receive a cemented lithium disilicate crown on a customized zirconia abutment (CEM) or a screw-retained crown with a directly veneered zirconia abutment (SCREW). At the screening visit, at crown insertion and at the 6-month follow-up, clinical parameters were measured at the implant and the contralateral tooth. Marginal bone levels, technical parameters, and esthetic outcomes were measured at the implants. At the 6-month follow-up, a microbiological test was performed and a soft tissue biopsy was harvested at the implants for histological analysis. Inflammatory cells and fibroblasts/-cytes were analyzed at the level of the sulcular epithelium, junctional epithelium, and connective tissue. The histological parameters were analyzed by means of a linear mixed model. RESULTS Thirty-three patients completed the study, and implant and crown survival rates were 100% at 6 months. Histologically, the number of inflammatory cells tended to be higher in group CEM ($p > 0.05$). Moreover, significantly less inflammatory cells and fibroblasts/-cytes were found in the sulcular epithelium compared to the junctional epithelium and supracrestal connective tissue ($p < 0.001$). Four patients were tested positive for periodontal marker pathogens at the 6-month follow-up, and three of them belonged to group CEM. From crown insertion to the 6-month follow-up, median marginal bone levels changed only minimally and measured 0.31 and 0.32 mm in group CEM and 0.47 and 0.36 mm in group SCREW, respectively. Clinical and esthetic parameters remained stable over time and were comparable between natural teeth and implants as well as between the groups. CONCLUSIONS Cemented reconstructions were associated with more inflammatory cells, and more patients were diagnosed with periodonto-pathogens. Both types of reconstructions resulted in similar radiological (marginal bone levels) and clinical outcomes (bleeding on probing and probing depth).

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Early histological, microbiological, radiological and clinical response to cemented and screw-retained all-ceramic single crowns

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Abstract

Objectives: To assess the early histological, microbiological, radiological and clinical response to cemented and screw-retained all-ceramic single-tooth implant-supported reconstructions.

Materials and methods: Patients with single-tooth implants were randomly allocated to receive a cemented lithium disilicate crown on a customized zirconia abutment (CEM) or a screw-retained crown with a directly veneered zirconia abutment (SCREW). At the screening visit, at crown insertion and at the 6-month follow-up, clinical parameters were measured at the implant and the contralateral tooth. Marginal bone levels, technical parameters and esthetic outcomes were measured at the implants. At the 6-month follow-up, a microbiological test was performed and a soft tissue biopsy was harvested at the implants for histological analysis. Inflammatory cells and fibroblasts/-cytes were analyzed at the level of the sulcular epithelium, junctional epithelium and connective tissue. The histological parameters were analyzed by means of a linear mixed model.

Results: Thirty-three patients completed the study and implant and crown survival rates were 100 % at 6 months. Histologically, the number of inflammatory cells tended to be higher in group CEM ($p>0.05$). Moreover, significantly less inflammatory cells and fibroblasts/-cytes were found in the sulcular epithelium compared to the junctional epithelium and supracrestal connective tissue ($p<0.001$). Four patients were tested positive for periodontal marker pathogens at the 6-month follow-up, three of them belonged to group CEM. From crown insertion to the 6-month follow-up, median marginal bone levels changed only minimally and measured 0.31 mm and 0.32 mm in group CEM and 0.47 mm and 0.36 mm in group SCREW respectively. Clinical and esthetic parameters remained stable over time and were comparable between natural teeth and implants as well as between the groups.

Conclusions: Cemented reconstructions were associated with more inflammatory cells and more patients were diagnosed with periodonto-pathogens. Both types of reconstructions resulted in similar radiological (marginal bone levels) and clinical outcomes (bleeding on probing, probing depth).

Introduction

Implant-supported single crowns are a predictable treatment option for replacing missing teeth due to their excellent long-term results (Jung, Zembic, Pjetursson, Zwahlen, Thoma, 2012). Porcelain-fused to metal crowns on titanium or gold abutments are well-documented in terms of long-term stability. However, in esthetic regions, these materials can compromise the esthetic treatment outcomes (Sailer, Zembic, Jung, Hammerle, Mattiola, 2007). All-ceramic crowns can overcome these issues offering superior esthetic, predominantly in sites with a thin mucosa (Dede, et al., 2016; Jung, et al., 2008). In addition, zirconia abutments could also have biological benefits regarding biocompatibility and reduced biofilm accumulation (Nakamura, Kanno, Milleding, Ortengren, 2010).

Implant-supported crowns can be fabricated as screw-retained or cemented crowns. Recent systematic reviews concluded that both types of reconstructions influenced the clinical outcomes in different ways, but none of the fixation methods was clearly advantageous over the other (Millen, Bragger, Wittneben, 2015; Sailer, Muhlemann, Zwahlen, Hammerle, Schneider, 2012). Cemented reconstructions were associated with more biological complications and these were considered to be more serious. This is mainly due to clinical evidence that excess cement in the peri-implant mucosa is a factor for increased biofilm accumulation. This in turn may cause peri-implant inflammation and peri-implant marginal bone loss (Staubli, Walter, Schmidt, Weiger, Zitzmann, 2016; Wilson, 2009). Screw-retained crowns exhibited more technical complications such as abutment screw-loosening. However, they offer the advantage of being more easily retrievable than cemented reconstructions (Jemt, 2009).

For zirconia abutments, it is unknown to date whether the use of cemented all-ceramic crowns or screw-retained implant crowns result in better clinical and biological outcomes. Cemented all-ceramic crowns on customized zirconia abutments are relatively well documented in clinical studies and have shown excellent clinical long-term outcomes and stable marginal bone levels (Canullo, 2007; Ekfeldt, Furst, Carlsson, 2011; Lops, Bressan, Chiapasco, Rossi, Romeo, 2013; Zembic, Bosch, Jung, Hammerle, Sailer, 2013; Zembic, Philipp, Hammerle, Wohlwend, Sailer, 2015).

For screw-retained crowns on zirconia abutments (directly veneered abutments), the data is scarce (Fabbri, et al., 2017; Thoma, et al., 2016).

The effect of the reconstructive materials i.e. zirconia, cement and veneering ceramics on the biology are even less investigated than the technical aspects (Linkevicius, Apse, 2008; Linkevicius, Vaitelis, 2015). Even if differences between materials are obvious, there is no clear evidence to date if they affect clinical outcomes such as soft tissue conditions and marginal bone level. The ideal surface quality remains a compromise between smoothness reducing biofilm accumulation and roughness increasing cell adhesion (Kim, Ko, Kye, Yang, 2014; Rutkunas, et al., 2015).

The aim of the present study was therefore, to compare cemented and screw-retained all-ceramic single-tooth implant-supported reconstructions in terms of histological, microbiological and early radiological and clinical outcome measures 6 months following the insertion of the final crowns.

Materials and methods

The study was designed as a randomized controlled clinical trial with two groups and a duration of 5 years. It was approved by the local ethical committee (No. 2012-0147) and registered at www.clinical-trials.gov (NCT01644630).

Study population

Informed consent was obtained from all patients. Patients were recruited consecutively for this trial between July 2012 and August 2014. The patients had to fulfill the following inclusion criteria: 18-80 years of age; one two-piece implant of 3.3 or 4.1 mm diameter (Straumann, Basel, Switzerland), successfully integrated (implant stability and < 1 mm marginal bone loss in the periapical radiograph) in the anterior maxilla or mandible (incisors, canines, premolars); at least one adjacent natural tooth present; implant position enabling both screw-retained and cemented crown. The exclusion criteria were smoking of more than 15 cigarettes per day, poor oral hygiene (plaque index over 30 %) or pregnancy. Periodontal diseases were treated before implant placement. At re-evaluation after periodontal therapy, residual pockets of < 5 mm were accepted to continue the treatment.

Randomization, allocation concealment

Patients receiving dental implants at the Clinic of Fixed and Removable Prosthodontics and Dental Material Science and meeting the inclusion criteria were screened and consecutively entered the clinical study at the time-point of the final impression. Following inclusion, patients were randomly allocated using a sealed envelope containing the group allocation according to a computer-generated list.

Group 1 (CEM): zirconia abutment with a veneered lithium disilicate crown

Group 2 (SCREW): customized zirconia abutment, directly veneered with veneering ceramic

Clinical and laboratory procedures

The impressions were taken digitally by using a scan body and an intraoral scanner (iTero, Straumann, Basel, Switzerland) or conventionally using a screw-retained implant pick-up and polyether impression material (Permadyne, 3M ESPE, Seefeld, Germany). Depending on the impression technique, either a printed or a plaster master cast was fabricated. The plaster models were digitized by the use of a desktop scanner (Imetric 3D, Courgenay, Switzerland). The zirconia abutments were designed and fabricated using the Straumann CARES system. For the screw-retained crowns, the zirconia abutments were designed in order to ideally support the veneering ceramic. For the cemented crowns, the crown margin was placed 0.5 mm submucosally and a try-in of the abutments in the patient's mouth was carried out in order to check or correct the position of the abutment margin in relation to the peri-implant mucosa. If the margin was placed too far submucosally, the abutment was discarded and refabricated. Subsequently, lithium disilicate crowns (IPS e.max press, Ivoclar Vivadent, Schaan, Liechtenstein) were fabricated and veneered manually for esthetic purposes. A bisque bake try-in was carried out for all reconstructions. The screw-retained crowns and the abutments for the cemented crowns were inserted by a torque of 35 Ncm indicated by the manufacturer. For the insertion of the cemented crowns, a retraction cord was placed. The lithium disilicate crowns were inserted after etching and silanization (Monobond S, Ivoclar Vivadent) with a universal resin cement (Rely X Unicem, 3M ESPE, Seefeld, Germany). The excess cement was removed meticulously by using carbon scalers, tactile and visual control. A periapical X-ray was taken in order to identify excess cement. The screw access holes of the screw-retained crowns were closed by using a teflon tape and composite (Tetric, Ivoclar Vivadent).
The measurements were performed 7-10 days after insertion of the final crown and again at 6 months.

Maintenance and follow-up

All patients received hygiene instructions and remained in a maintenance program with dental hygiene visits twice a year. Visits contained plaque record, probing depth, bleeding on probing measurements and supragingival cleaning.

Microbiological testing

At the 6-month follow-up, microbiological samples were harvested at the mesial and distal aspects of the implants using a multiplex real-time polymerase chain reaction test (IAI Pado Test, Institut IAI, Zuchwil, Switzerland). According to the manufacturer's instructions, the supragingival biofilm was first removed with a curette. Sterile paper points were inserted into the sulcus in order to collect a subgingival sample. Bacterial ribosomal 16S rRNA was detected and allowed quantification of total bacterial load and four periodontal marker pathogens (*Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola*). The values were calculated based on the estimated ribosome content per bacterial cell.

Harvesting of biopsies

At the 6-month follow-up, a semilunar shaped biopsy of the peri-implant mucosa at the palatal or lingual aspect of the implants was harvested (only if at least 2 mm keratinized tissue was present). For that purpose, a sulcular incision along the abutment was connected to a para-marginal incision (at a distance of 2 mm from the sulcus) at the disto-lingual and mesio-lingual line angles. The vertical dimension extended from the mucosal margin to the bone crest.

Histological preparation and analyses

The biopsies were fixed in 4 % buffered formalin for at least 48 hours prior to histological preparation. The specimens were dehydrated and infiltrated with xylol and paraffin (Paraffin at 60° Celsius). Subsequently, specimens were embedded in paraffin and cut into 2-5 µm thick sections using a microtome (MICROM, Medite GmbH, Dietlikon, Switzerland). All sections were stained with Hematoxylin-eosin (HE). Light microscopic evaluation of all sections was performed by a blinded laboratory technician using an optical microscope (Leica CTR600, Leica, Wetzlar, Germany) at a 200 x magnification (Figure 1a). An image editing software (Adobe Photoshop CS6 extended, Adobe Systems, San José, CA, USA) was used to mark inflammatory cells,

fibroblasts/-cites, epithelium and background. The percentage of the area of all subgroups was then calculated for a semi-quantitative analysis (LAS V4.3, Leica, Wetzlar, Germany) (Figure 1b+c). Three sectors of interest at different levels were defined: sulcular epithelium (SE), junctional epithelium (JE), supracrestal connective tissue (CT) (Figure 1d).

Marginal bone level

Standardized single-tooth radiographs were taken at crown insertion and at the 6-month follow-up. The x-rays were digitized and the bone level was measured at 10x to 15x magnification. The distance between the threads (0.8 mm) of the implant was used as reference for adjusting the scale (ImageJ, National Institute of Health, Bethesda, MD, USA). The distance between the implant shoulder and the bone crest was assessed at the mesial and distal aspect of each implant and mean values were calculated. All measurements and calculations were done by a blinded examiner not part of the surgical and/or prosthetic procedures.

Technical outcome measures

Technical aspects were recorded after crown insertion and 6 months according to modified USPHS (United States Public Health Service) criteria. The crowns were examined for catastrophic fracture, fracture of the veneering ceramic, abutment screw fracture or loosening, occlusal wear, marginal adaption and decementation. The parameters were recorded as alpha (A), bravo (B), charlie (C) or delta (D).

Clinical and esthetic parameters

At the screening visit, crown insertion and at the 6-month follow-up, the plaque control record (PCR) (O'Leary, Drake, Naylor, 1972), bleeding on probing (BOP) and probing depth (PD) were assessed at six sites of the implants and neighboring teeth by means of a periodontal probe (PCB 12, Hu-Friedy, Leimen, Germany). The width of keratinized tissue (KT) was assessed at the buccal mid-facial aspect of the implant and neighboring teeth. The mucosal thickness (MT) around implants sites was assessed to the nearest 0.5 mm at a level 1 mm apically from the

mucosal margin using an endodontic file. In addition, the mid-facial clinical crown height was measured by means of a periodontal probe and the gingival recession (REC) from crown insertion to 6 months was calculated. The mesial and distal height of the papillae was assessed using the modified papilla Index (Jemt, 1997).

Color measurements

A spectrophotometric measurement was carried out at the buccal peri-implant mucosa and at the gingiva of the contralateral natural control tooth, 1 mm beneath the crown margin (MHT Spectrophotometer, Niederhasli, Switzerland). Values for Lightness (L), color-opponent dimension with a position between red/magenta and green (a) and color-opponent dimension with a position between yellow and green (b) values were measured. The color difference ΔE between the implant and the contralateral natural control tooth was calculated applying the following formula: $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$.

Statistical analysis

The power analysis was carried out for marginal bone levels based on a 5-year study using two-piece dental implants revealing a standard deviation of marginal bone loss of 0.46 mm (Palmer, Palmer, Smith, 2000). A sample size of 15 in each group will have 80 % power to detect a difference in means of -0.5 mm. When a patient drop-out rate of 10 % is assumed, the target sample size in each group increases to 17.

All parameters were analyzed descriptively, calculating the mean, standard deviation, median, minimum, maximum and 1st and 3rd quartile. Two separate linear mixed models were calculated for the histological target parameters "inflammatory cells" and "fibroblasts/-cytes", respectively. Each time, the explanatory variables group (CEM/SCREW) and region (JE, SE, CT) were taken as fixed effects and the patient as a random effect. Posthoc pairwise comparisons were performed according to Tukey . The level of significance was set to α= 5 %. All statistical analyses and plots were done with the statistical software R (R Core Team, 2015), including the packages ggplot2 (Wickham, 2009) and lmerTest (Kuznetsova, Brockhoff, Christensen, 2016).

Results

Patient demographics and implant characteristics

Thirty-four patients were originally included in the study, examined at the screening visit and randomized. One patient of group CEM was excluded because the abutment had been modified with veneering ceramic in the subgingival part due to a misunderstanding with the technician. Another patient (group SCREW) attended the screening visit only and was therefore excluded. One more patient was recruited and randomized, resulting in thirty-three included patients (16 in group SCREW, 17 in group CEM), which were examined over the complete observation period. Patient characteristics are summarized in Table 1. Between the screening visit and the 6-month follow-up, no implants or crowns were lost, resulting in a 100% survival rate on both, the implant and restorative level.

Histologic evaluation

Eight patients in each group had enough keratinized tissue surrounding the implant and agreed for the harvesting of a biopsy at the 6-month follow-up. Variations in between patients and groups were relatively high. The number of inflammatory cells was higher in group CEM in all three regions of interest, but the differences were not statistically significant ($p>0.05$). Comparing the regions of interest, the amount of inflammatory cells as well as fibroblasts/-cytes was significantly lower in the SE compared to the JE and CT ($p<0.001$ for both groups and both parameters). Figures 2a+b represent an area with a low respectively a high amount of inflammatory cells. The results of the histological analysis are presented in Figure 3 and the descriptive data are summarized in Table 2.

Bacterial testing

Four patients (12.1 % of all patients) were tested positive for periodontal marker pathogens at the 6-month follow-up. Three of these patients belonged to group CEM. Two patients were tested positive for *P. gingivalis, T. forsythia, T. denticola* whereas two patients were positive for T.

denticola only. No patient was tested positive for *A. actinomycetemcomitans*. The total bacterial loads are reported in Table 2.

Marginal bone level

The median marginal bone levels (MBL) at crown insertion were 0.31 mm (Q1 = 0.13; Q3 = 0.83) in group CEM and 0.47 mm (Q1 = 0.25; Q3 = 0.70) in group SCREW. At the 6-month follow-up, the MBL was located at 0.32 mm (Q1 = 0.12; Q3 = 0.87) (CEM) and 0.36 mm (Q1 = 0.21; Q3 = 0.61) (SCREW) (Table 3).

Clinical parameters

The values for PCR, PD, KT remained stable over time and were comparable between implants and natural control teeth. A slight temporary increase in median BOP was detected, from 0 % (Q1 = 0; Q3 = 4, CEM) and 8 % (Q1 = 0; Q3 = 33, SCREW) at screening (with the healing abutment in situ) to 33 % (Q1 = 0; Q3 58, CEM) and 17 % (Q1 = 0; Q3 = 33, SCREW) after crown insertion. However, at the 6-month follow-up, BOP values decreased to 17 % (Q1 = 4; Q3 = 50, CEM) and 17 % (Q1 = 0; Q3 = 33, SCREW) (Table 3).

Technical outcome measures

USPHS: Two minor chippings (group CEM) occurred immediately after crown insertion and were noted at the baseline visit. They were polished and were no more recorded at 6 months. Only approximately half of the contact points were rated alpha after crown insertion and at 6 months. Regarding marginal adaptation, one crown in the cemented group had a detectable cementation gap at both visits. All other parameters, not specifically mentioned were rated alpha at both time-points.

Clinical and esthetic parameters

All clinical and esthetic parameters including mucosal thickness, papilla index, crown height and spectrophotometric measurements are reported in (Table 4).

Discussion

The present 6-month follow-up of screw-retained, directly veneered or cemented crowns on customized zirconia implant abutments revealed i) a tendency to a lower number of inflammatory cells for screw-retained reconstruction ii) significantly less inflammatory cells and fibroblasts/-cytes in the sulcular epithelium compared to the other regions iii) stable marginal bone levels for both reconstruction types iv) no differences in clinical, esthetic or technical parameters.

The present study is based on the comparison of two frequently applied clinical concepts. Zirconia abutments receiving an all-ceramic crown by means of adhesive cementation have become an established treatment concept for the esthetic zone (Bidra, Rungruanganunt, 2013). Moreover, data on ceramic crowns made of lithium disilicate demonstrated promising clinical results (Joda, Ferrari, Bragger, 2017; Simeone, Gracis, 2015) All-ceramic single crowns with a zirconia framework and a feldspathic veneering tend to show a higher chipping rate compared to porcelain fused to metal crowns or monolithic crowns (Schwarz, Schroder, Hassel, Bomicke, Rammelsberg, 2012). In addition, screw-retained reconstructions exhibit a less complex fabrication with a directly veneered zirconia abutment. A comparison of the two concepts does not only imply differences in terms of the type of retention, but further involve differences in terms of fabrication and material composition. As such, technical outcomes can be assessed comparing the two concepts, but are limited since the components of the two types of reconstructions differ to some extent. Apart from technical outcome measures, scientific data based on preclinical and clinical studies indicated that the type of retention and the material properties further influence biological outcomes. Most frequently, biological outcomes are reported based on clinical parameters (BOP, PD) and radiological assessments and can be supplement by histologic and microbiological outcome measures.

Based on soft tissue biopsies harvested 6 months post loading with final reconstructions, the number of inflammatory cells tended to be higher in the group with cemented crowns in all three regions (JE, SE and CT). Moreover, significantly less inflammatory cells were present in the SE compared to the JE and CT. Three of four patients were tested positive for a periodontal marker

pathogen belonged to group CEM, supporting the tendency as seen in the histomorphometric results.

The design and fabrication of the two types of reconstructions has substantial differences. This results in two reconstructions differing within the soft tissue transition zone in terms of surface material, roughness and the presence of a cement gap. In the cemented group, soft tissues are mainly attached to the zirconia abutment surface. The surface roughness and structure remain unchanged during the fabrication in the dental lab and are based on the centralized manufacturing process. The transition between the implant abutment (zirconia) and the cemented crown (lithium disilicate crown with a glazed surface) is located slightly submucosally. The screw-retained reconstructions are veneered in the subgingival part to some extent, usually extending 1-3mm more apically from the abutment shoulder. The extent of veneering depends on the height and width of the emergence profile. Areas closer to the implant shoulder remain un-veneered, however.

Commonly used veneering materials have average roughness (Ra) values between 0.143 to 0.150 µm (Tang, et al., 2015). The number of firings did influence the Ra values only minimally in the mentioned study, but aging increased the Ra value up to 0.359 µm. Compared to the surface roughness of the ceramics, the applied self-adhesive cement has a way higher roughness with values of 4.4 µm (Cresti, Itri, Rebaudi, Diaspro, Salerno, 2015). Hence, the harvested soft tissues were either attached to a median rough zirconia abutment plus a rough cementation gap (CEM) or mainly to a smooth veneering ceramic and a median rough zirconia abutment (SCREW).

Furthermore, studies have shown that excess cement cannot be removed completely, especially in the case of adhesive cementation (Agar, Cameron, Hughbanks, Parker, 1997; Sancho-Puchades, et al., 2017). This in turn is further supported by clinical studies demonstrating adverse effects of cement on biological outcomes (Staubli, et al., 2016; Wilson, 2009). Interestingly, in the present study, the patient with a visible cementation gap in the x-ray according to the USPHS criteria was tested positive on the mesial and distal aspect on *T. forsythia, P. gingivalis and T. denticola*. Unfortunately, this patient could not be included for biopsy harvesting, the results would possibly have been further correlating. In vitro, restoration

margin morphology and interface roughness have affected bacterial colonization (Anami, et al., 2012).

It is speculated that the observed differences were mainly associated with the cement itself and with possible gaps or clinically undetected excess cement. Whether or not histologic and microbiologic outcome might serve as an early indicator of adverse biologic reactions at implant sites, has to be further evaluated. Outcome measures such as PD and marginal bone levels were not affected up to 6 months by the observed higher rate of inflammatory cells. Follow-up examinations using the same patient pool will provide evidence on the long-term effect and influence of the type of retention on biological and technical outcomes in the future.

Limitations of the present study predominantly include a relatively short observation period (for marginal bone levels and technical outcomes), the number of biopsies (sample size was calculated for marginal bone level changes) and the two types of reconstructions that differed not only by the type of retention (limitation does only apply to biological outcomes). In terms of microbiological testing, the qualitative results are difficult to interpret due to the small sample size (only four positive results), and the quantitative results are an estimate and influenced by several clinical factors such as supragingival plaque removal and amount of sulcular fluid. Furthermore, microbiological data after crown insertion would have been an ideal baseline to compare the findings of the 6-month time-point. Only two-piece implants from one company were included. This was to standardize the procedures since it is known that the implant design influences marginal bone level changes and other parameters. Since in the esthetic area, anatomical dimensions vary, two implant diameters were allowed to be placed.

Conclusions

Cemented reconstructions were associated with more inflammatory cells and more patients in the CEM group were diagnosed with periodonto-pathogens. Statistically, no significant differences were observed between the two groups CEM and SCREW. Both types of reconstructions resulted in similar radiological (marginal bone levels) and clinical outcomes (bleeding on probing, probing depth).

Figure Legend

Figure 1 a-d A region of interest with a high amount of inflammatory cells is shown (a). Inflammatory cells were marked red, fibroblasts/-cites were marked yellow, and epithelium was marked blue (b). The remaining background, mainly connective tissue, was marked green. The percentage of the area of every color was then calculated (c). Three regions were evaluated on each biopsy (d), the sulcular epithelium (SE), the junctional epithelium (JE) and the supracrestal connective tissue (CT).

Figure 2a Supracrestal connective tissue with a low amount of inflammatory cells.

Figure 2b Junctional epithelium with a high amount of inflammatory cells.

Figure 3 Boxplot diagram representing the results of the histological analysis. Data was log-transformed and both endpoints (the amount of inflammatory cells and fibroblasts/-cytes) were analyzed by a linear mixed model. While the tendency of higher counts in the CEM group could not be corroborated statistically, a significant difference between the regions SE and JE as well as between SE and CT was found ($p<0.001$ for both comparisons and both endpoints). CEM = cemented group; SCREW = screw-retained group; SE = sulcular epithelium; JE = the junctional epithelium; CT = supracrestal connective tissue; ns = not statistically significant.

Table 1 Patient characteristics including gender, age and surgical site. CEM = cemented group; SCREW = screw-retained group; Q1 = 25% quartile; Q3 = 75% quartile.

Table 2 Histological and microbiological data at the 6-month follow-up. N = number; SD = standard deviation; Min = minimum; Q1 = 25% quartile; Q3 = 75% quartile; Max = maximum; SE = sulcular epithelium; JE = the junctional epithelium; CT = supracrestal connective tissue.

Table 3 Clinical parameters at the screening visit (Screening), at crown insertion and at the 6-month follow-up. N = number; SD = standard deviation; Min = minimum; Q1 = 25% quartile; Q3 = 75% quartile; Max = maximum; BOP = Bleeding on probing; PCR = Plaque record index; PD = Probing depth; KT = Keratinized tissue; Tooth = Contralateral tooth; MBL = marginal bone level, mesial and distal combined.

Table 4 Esthetic parameters at crown insertion and at the 6-month follow-up. N = number; SD = standard deviation; Min = minimum; Q1 = 25% quartile; Q3 = 75% quartile; Max = maximum; Jemt = Modified papilla index; MT = Mucosa thickness.

Acknowledgements and conflict of interest

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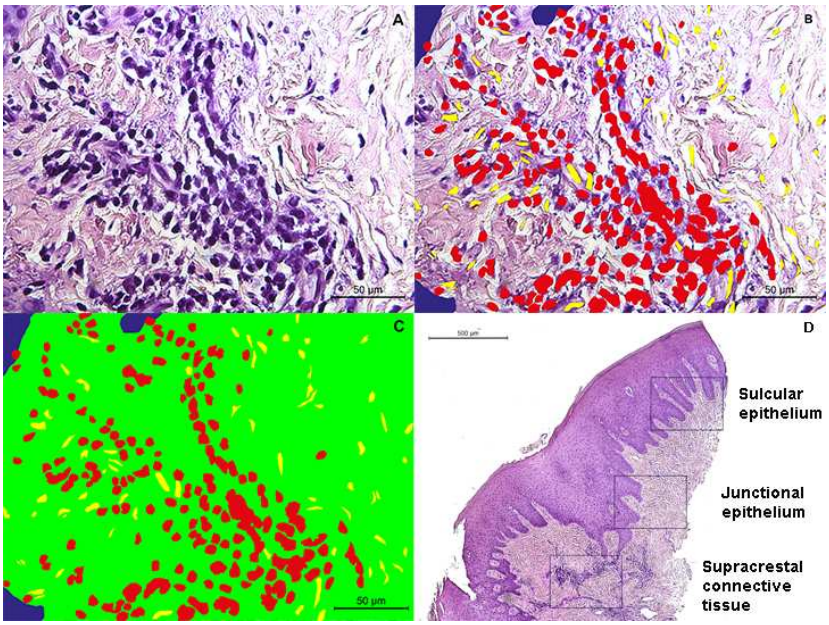

Figure 1

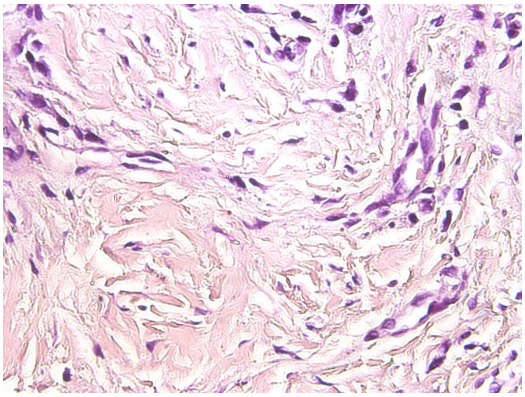

Figure 2a

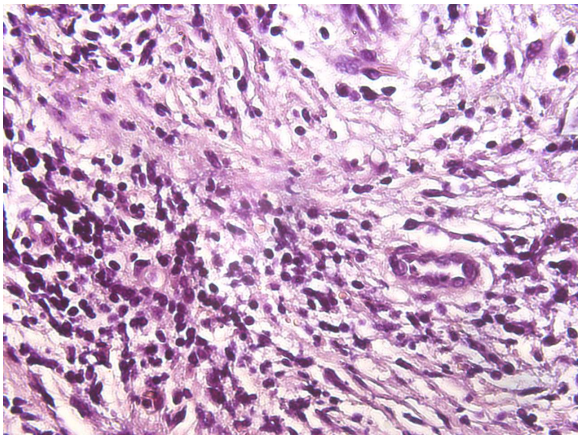

Figure 2b

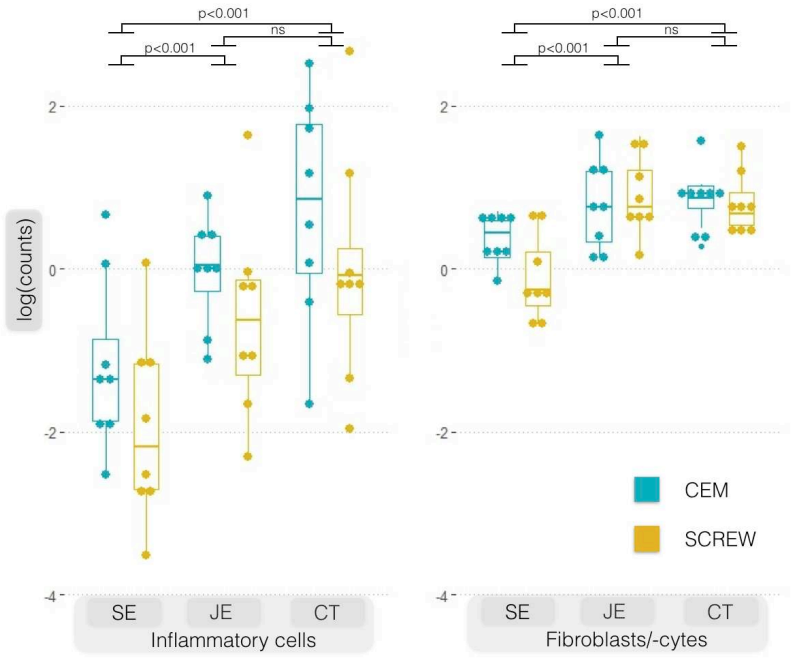
p<0.001
ns
p<0.001
p<0.001
ns
p<0.001
log(counts)
2
0
-2
-4
SE
JE
CT
Inflammatory cells
Fibroblasts/-cytes
CEM
SCREW

Figure 3

Table 1. Patient characteristics

		SCREW	CEM
N		16	17
Gender	male	6	7
	female	10	10
Age	median	50.38	53.34
	Q1;Q3	41.11;57.80	40.63;62.20
Implant site characteristics	maxilla	14	14
	mandible	2	3
	incisors	5	6
	canines	2	2
	premolars	9	9

Table 2. Histological and microbiological outcomes

		Cemented								Screw-retained							
	Variable	N	Mean	SD	Min	Q1	Median	Q3	Max	N	Mean	SD	Min	Q1	Median	Q3	Max
6-month follow-up	Inflammatory cells, SE (%)	8	0.53	0.61	0.08	0.15	0.26	0.50	1.94	8	0.26	0.33	0.03	0.07	0.12	0.31	1.08
	Inflammatory cells, JE (%)	8	1.16	0.64	0.33	0.81	1.04	1.49	2.47	8	1.09	1.56	0.10	0.28	0.57	0.88	5.14
	Inflammatory cells, CT (%)	8	4.02	3.95	0.19	0.98	2.48	5.98	12.52	8	2.71	4.55	0.14	0.63	0.92	1.52	14.50
	Fibroblasts/-cytes, SE (%)	8	1.49	0.39	0.86	1.15	1.57	1.81	2.02	8	1.02	0.55	0.51	0.64	0.77	1.28	2.00
	Fibroblasts/-cytes, JE (%)	8	2.49	1.29	1.14	1.41	2.13	3.34	5.14	8	2.71	1.25	1.19	1.89	2.16	3.39	5.11
	Fibroblasts/-cytes, CT (%)	8	2.57	0.99	1.32	2.13	2.41	1.29	4.84	8	2.38	0.97	1.51	1.71	1.96	2.61	4.52
	Total bacterial load (millions)	15	19.68	9.83	3.18	14.79	19.19	20.55	43.05	15	18.83	9.36	4.32	11.67	17.40	24.78	35.27

Table 3. Clinical and radiological outcomes

			Cemented								Screw-retained							
	Variable		N	Mean	SD	Min	Q1	Median	Q3	Max	N	Mean	SD	Min	Q1	Median	Q3	Max
Screening	BOP (%)	Implant	14	8	19	0	0	0	4	67	10	15	18	0	0	8	33	50
		Tooth	16	7	9	0	0	4	15	25	16	9	15	0	0	0	21	42
	PCR (%)	Implant	14	2	9	0	0	0	0	33	10	0	0	0	0	0	0	0
		Tooth	15	0.14	0.14	0.00	0.00	0.08	0.33	0.33	16	0.06	0.09	0.00	0.00	0.00	0.08	0.33
	PD (mm)	Implant	14	2.54	0.72	1.67	2.00	2.42	2.88	4.33	10	3.23	0.61	2.33	2.96	3.00	3.92	4.17
		Tooth	15	2.27	0.45	1.50	1.83	2.25	2.42	3.25	16	2.34	0.43	1.42	2.02	2.54	2.73	2.75
	KT (mm)	Implant	14	3.21	1.05	2.00	2.00	3.50	4.00	5.00	10	4.15	1.16	3.00	3.00	4.00	5.25	6.00
		Tooth	16	3.19	0.98	1.00	2.50	3.00	3.50	5.00	12	3.54	1.47	1.00	2.13	4.00	4.50	5.50
Crown insertion	BOP (%)	Implant	17	33	27	0	0	33	58	67	16	17	15	0	0	17	33	33
		Tooth	16	15	15	0	0	17	25	58	16	24	15	0	8	25	33	50
	PCR (%)	Implant	17	10	20	0	0	0	8	67	16	5	10	0	0	0	13	33
		Tooth	16	20	22	0	0	13	40	67	16	19	18	0	0	17	31	58
	PD (mm)	Implant	17	2.89	0.51	2.00	2.50	2.83	3.33	3.67	16	2.98	0.58	2.00	2.67	2.92	3.17	4.17
		Tooth	16	2.25	0.46	1.67	1.83	2.25	2.56	3.50	16	2.31	0.41	1.50	2.02	2.42	2.67	2.92
	KT (mm)	Implant	17	3.00	1.41	1.00	2.00	3.00	4.00	6.00	16	3.91	0.93	2.00	3.00	4.00	5.00	5.00
		Tooth	17	3.18	1.46	1.00	2.25	2.50	4.00	6.00	16	3.78	1.17	1.50	2.75	4.00	4.50	5.50
	MBL (mm)	Implant	16	0.53	0.55	0	0.13	0.31	0.83	1.79	16	0.55	0.46	0	0.25	0.47	0.70	1.73
6-month follow-up	BOP (%)	Implant	16	31	29	0	4	17	50	83	15	24	21	0	0	17	33	67
		Tooth	15	17	19	0	0	8	25	58	15	13	13	0	0	8	17	50
	PCR (%)	Implant	16	11	17	0	0	0	29	50	15	7	14	0	0	0	0	33
		Tooth	15	17	19	0	0	17	25	58	15	13	15	0	0	8	18	50
	PD (mm)	Implant	16	3.00	0.74	1.33	2.71	3.00	3.63	4.33	15	3.08	0.51	2.00	2.83	3.00	3.50	3.83
		Tooth	15	2.32	0.34	1.58	2.08	2.25	2.67	2.83	15	2.46	0.41	1.92	2.17	2.33	2.67	3.33
	KT (mm)	Implant	16	3.25	1.13	1.00	2.25	3.00	4.00	5.00	14	3.64	1.01	2.00	3.00	3.50	4.00	6.00
		Tooth	16	3.16	1.36	1.00	2.13	3.00	4.50	5.50	14	3.36	1.12	1.00	2.88	3.50	4.00	5.50
	MBL (mm)	Implant	14	0.55	0.50	0.03	0.12	0.32	0.87	1.55	15	0.48	0.38	0.06	0.21	0.36	0.61	1.30

Table 4. Esthetic outcomes

		Cemented								Screw-retained							
	Variable	N	Mean	SD	Min	Q1	Median	Q3	Max	N	Mean	SD	Min	Q1	Median	Q3	Max
Crown insertion	Jemt mesial	17	1.65	0.79	0.00	1.00	2.00	2.00	3.00	16	1.69	0.70	1.00	1.00	2.00	2.00	3.00
	Jemt distal	17	1.35	0.70	0.00	1.00	1.00	2.00	3.00	15	1.40	0.63	1.00	1.00	1.00	2.00	3.00
	Crown height (mm)	17	9.24	1.21	6.50	9.00	9.00	10.00	12.00	16	9.03	2.04	6.00	7.03	9.25	10.38	13.00
	MT (mm)	17	3.38	0.99	1.50	2.50	4.00	4.00	5.00	16	4.06	1.06	2.00	3.25	4.00	5.00	5.50
	Color difference (ΔE)	13	5.51	1.56	3.80	3.86	5.55	6.58	9.18	11	7.42	5.05	3.06	4.43	5.60	7.74	19.33
6-month follow-up	Jemt mesial	16	1.75	0.93	0.00	1.00	2.00	2.75	3.00	14	1.79	0.70	1.00	1.00	2.00	2.00	3.00
	Jemt distal	16	1.44	0.81	1.00	1.00	1.00	1.75	3.00	14	1.36	0.63	1.00	1.00	1.00	2.00	3.00
	Crown height (mm)	16	9.00	1.90	4.00	9.00	9.00	9.50	13.50	14	9.00	1.89	6.50	7.00	9.25	10.00	13.00
	MT (mm)	15	3.40	0.60	2.50	3.00	3.00	4.00	4.00	14	4.11	0.86	2.50	3.38	4.00	5.00	5.00